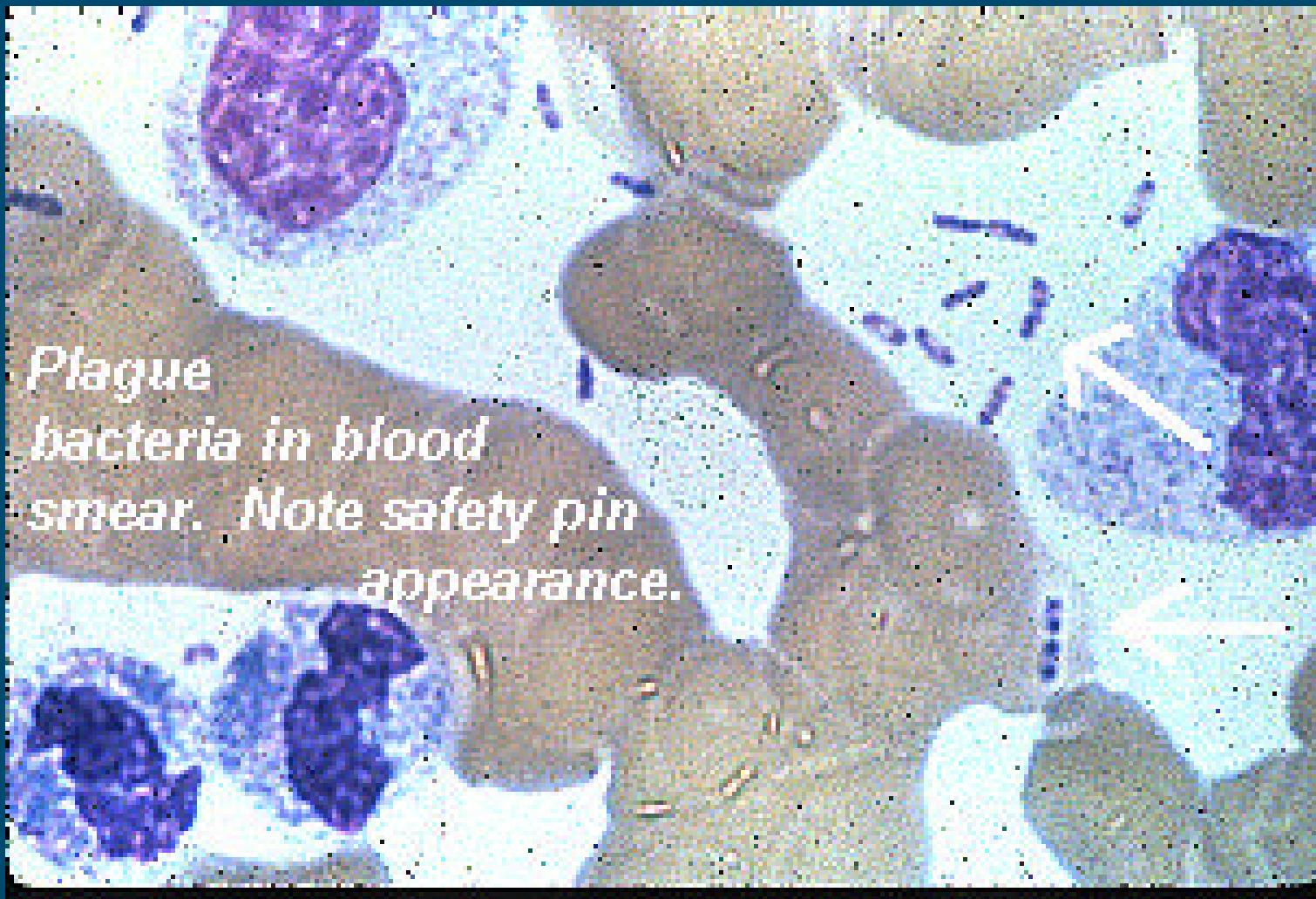


The role of antibodies and cell-mediated immunity conferring protection against pathogens

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Plague bacteria in blood

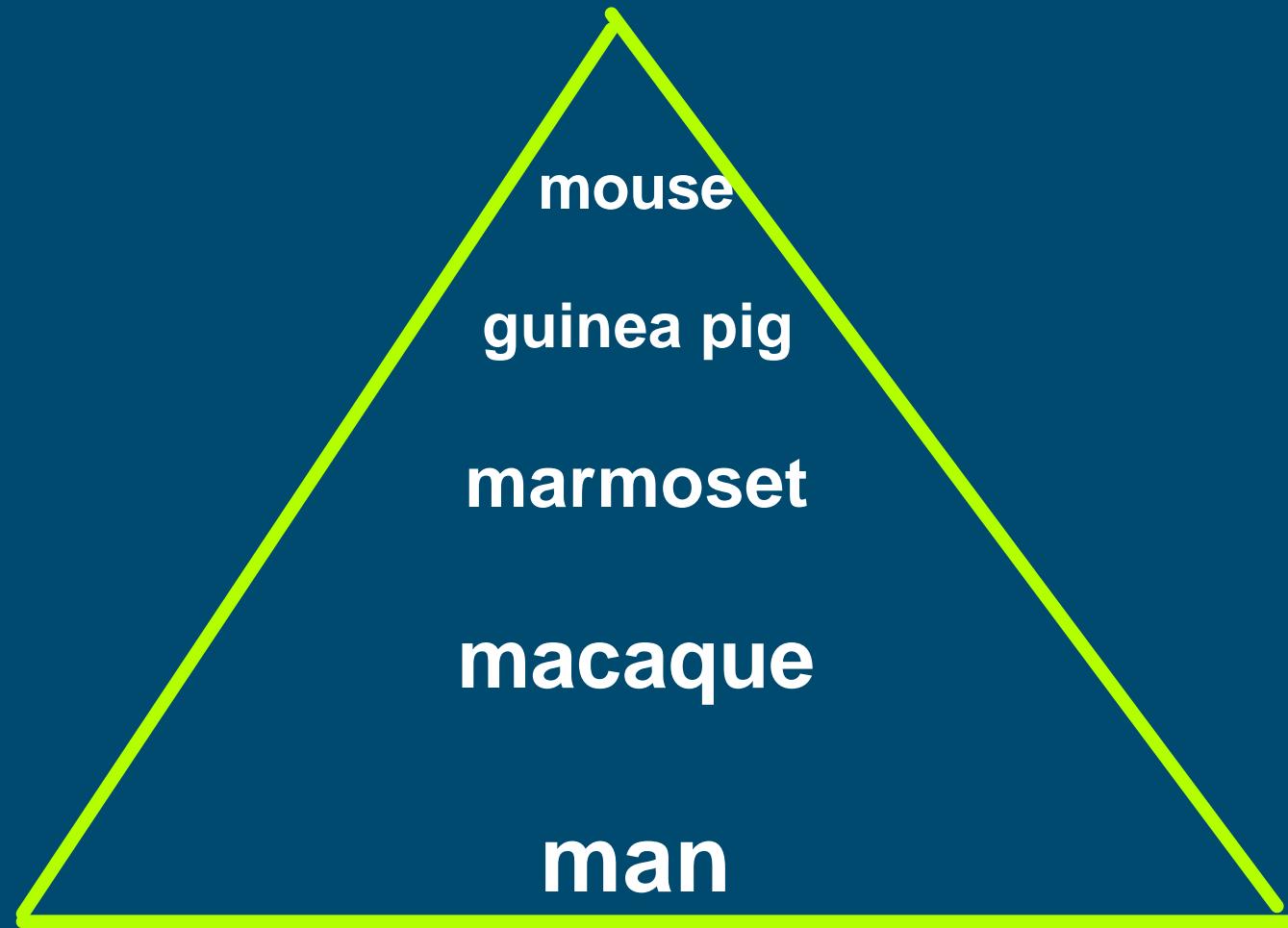


*Plague
bacteria in blood
smear. Note safety pin
appearance.*

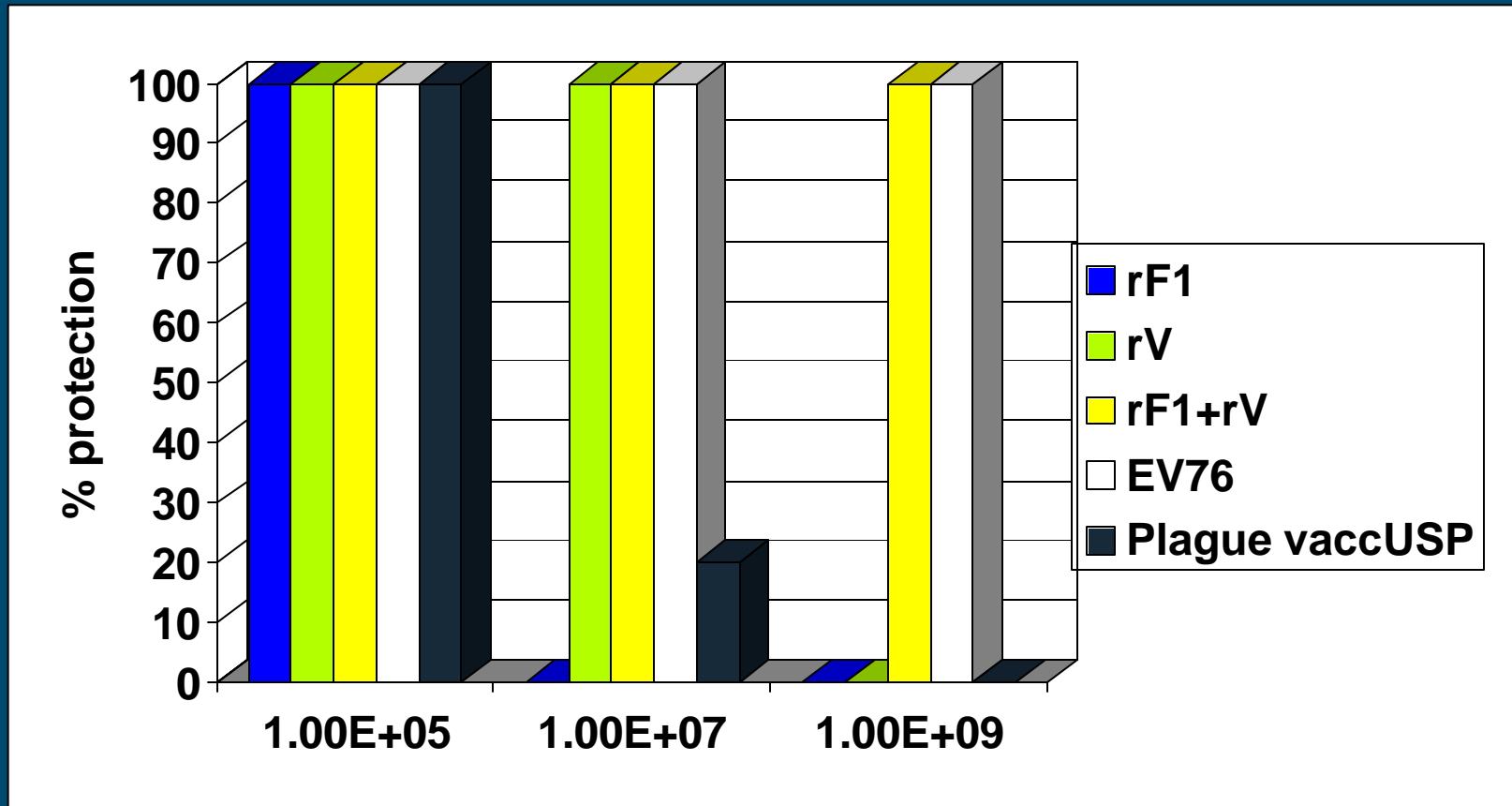
Immune response

- Protection against plague will depend on countering the bacterium and its virulence factors
- Inducing an appropriate immune response in the host
- What are the opportunities for intervention?
 - ab
 - CMI
- utility of animal models to elucidate immune correlates

Role of antibody and CMI



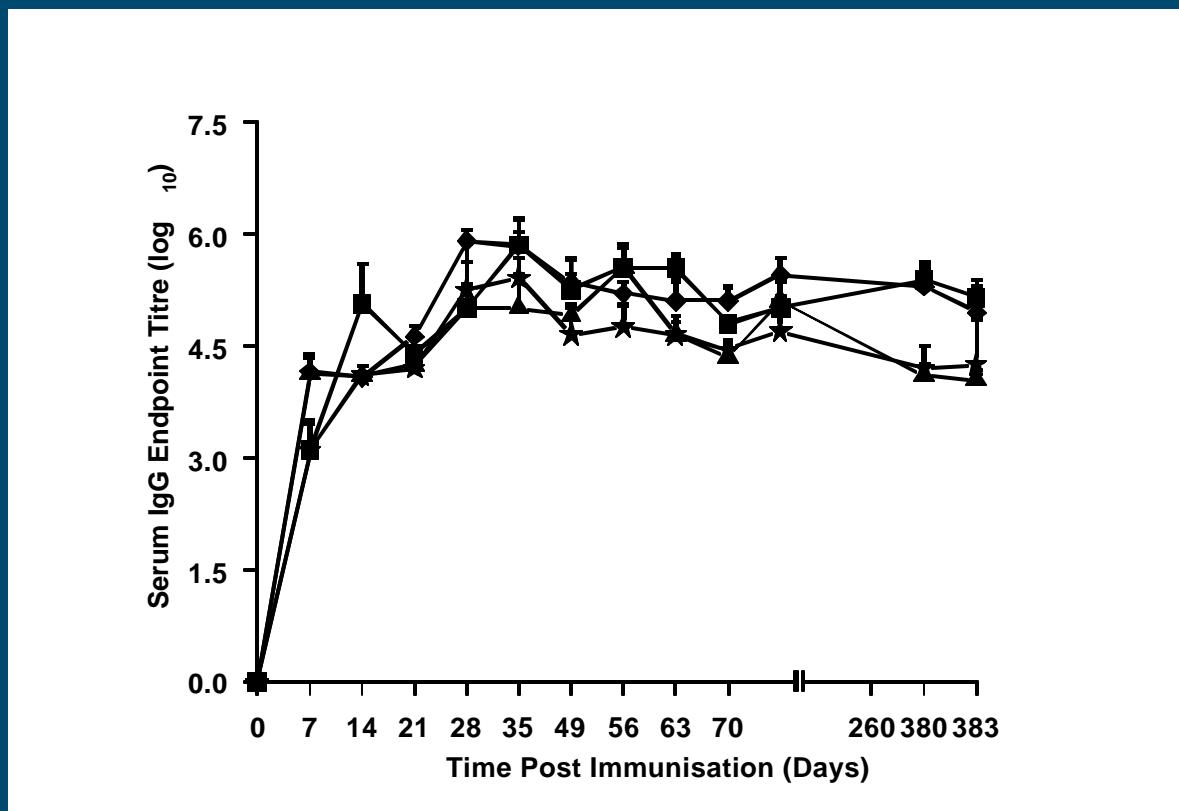
Additive protective effect of rF1+rV against sub-cutaneous challenge (female Balb/c mouse) Williamson et al 1995



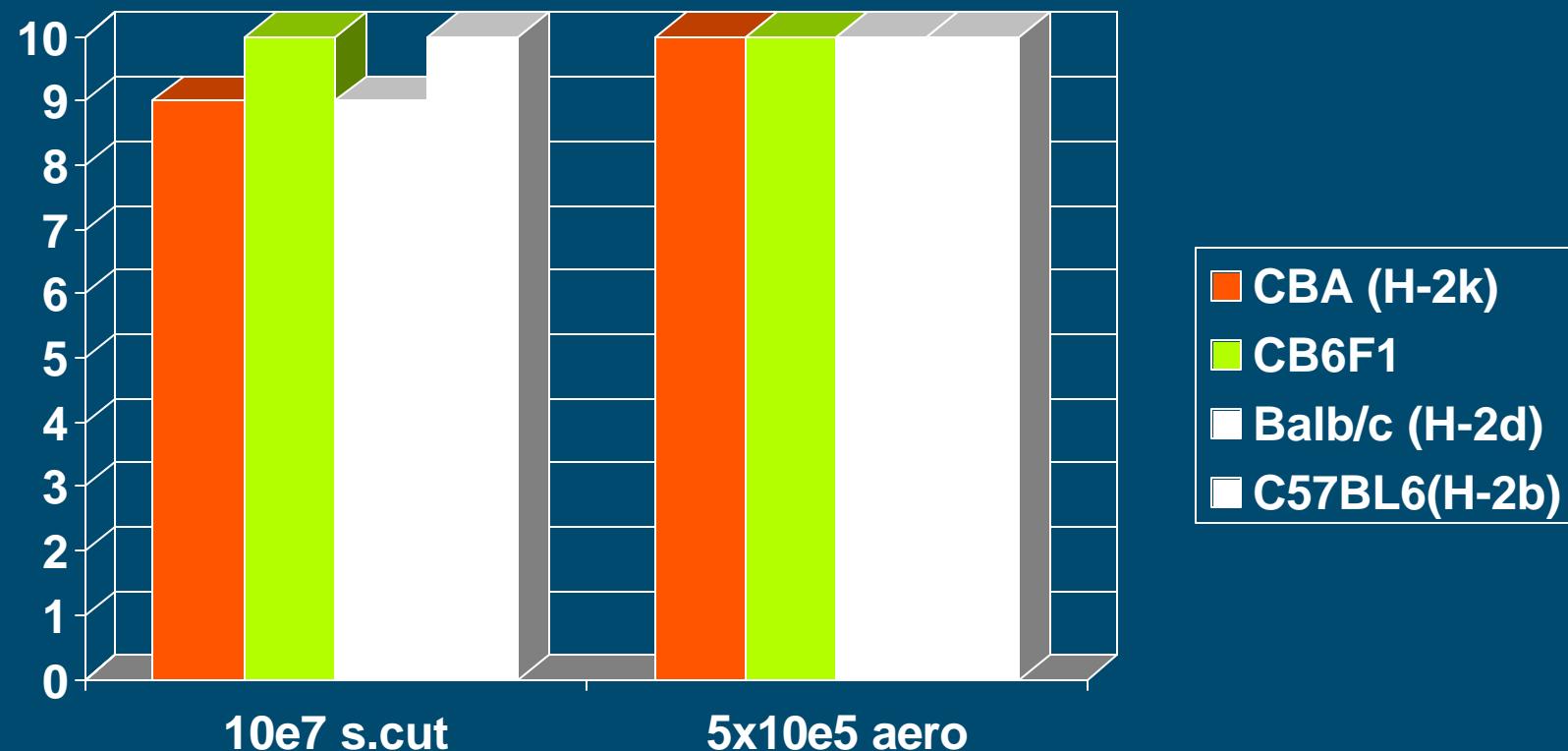
Antibody response

- characterise the response: kinetics, quantity
- seek similarities between animal models
- relate antibody characteristics to protective efficacy
- rationale for extrapolation from models to man

IgG titre to V antigen in female mice (day 0,21 i.m.) and boosted i.p. (day 380) to stimulate a memory response. The group mean endpoint titres are shown BALB/c ●, C57BL6 ?, CBA ▲, CB6F1 ▨ (Jones et al Vaccine 2001, 19: 358-366)



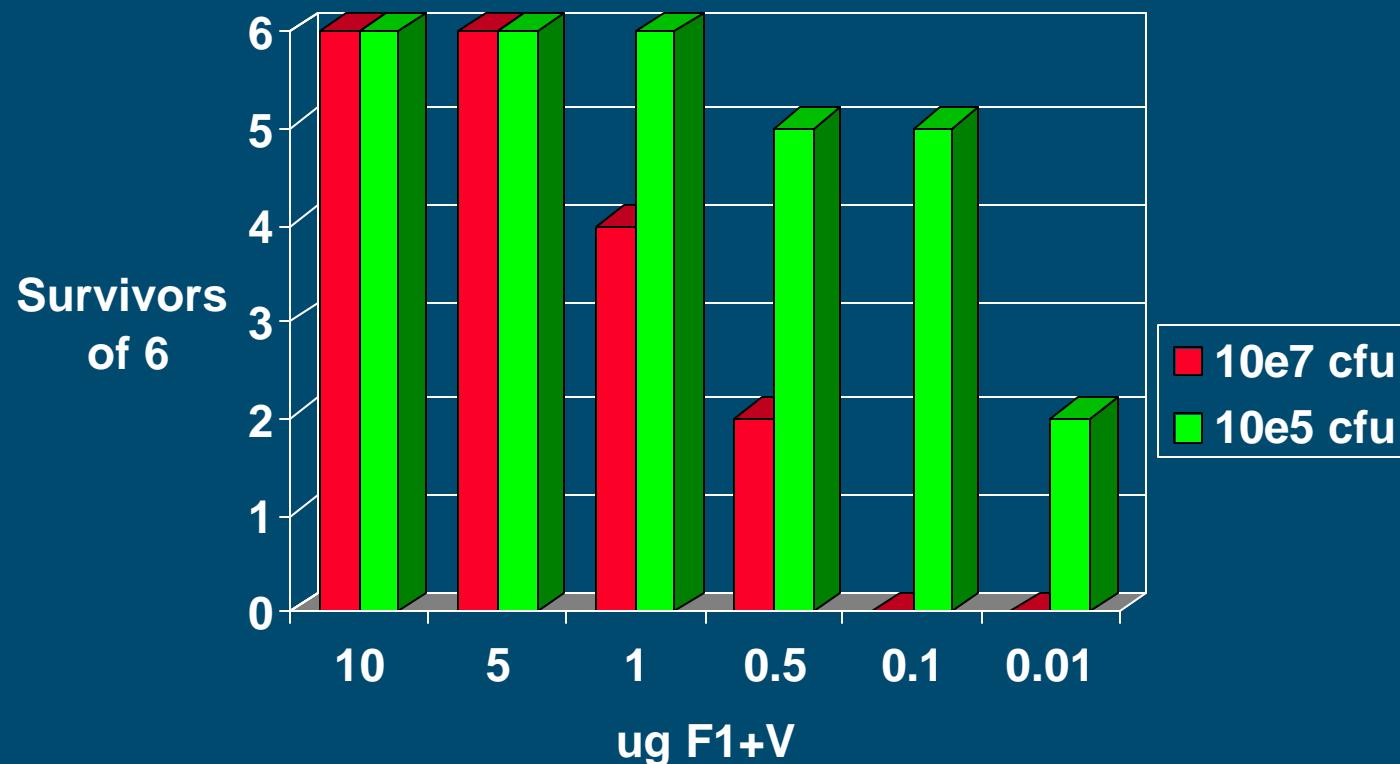
Efficacy of rF1+rV against challenge in female mice (day +80) challenge



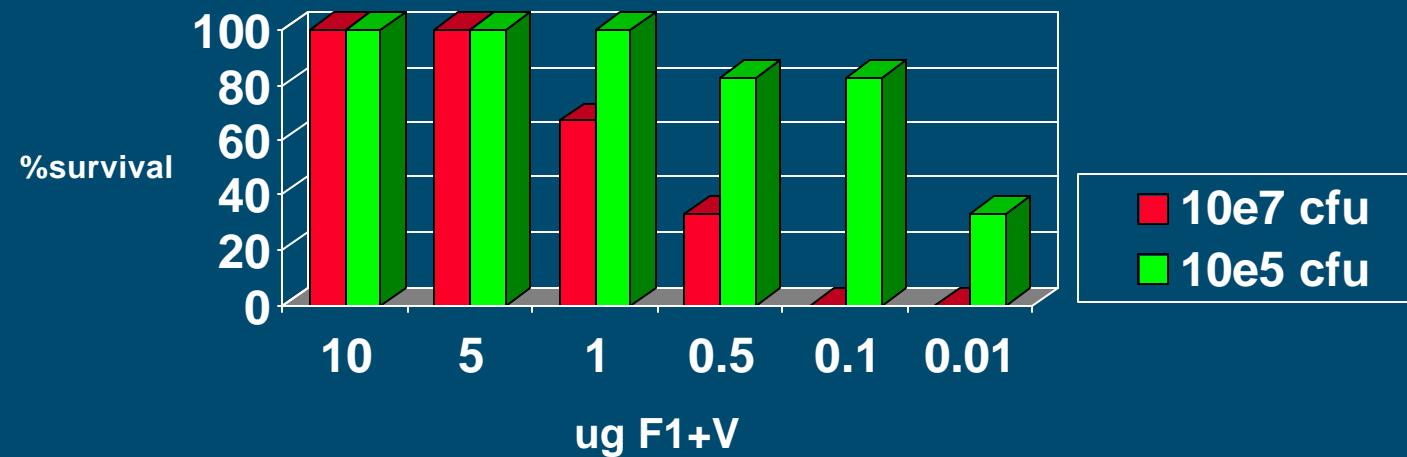
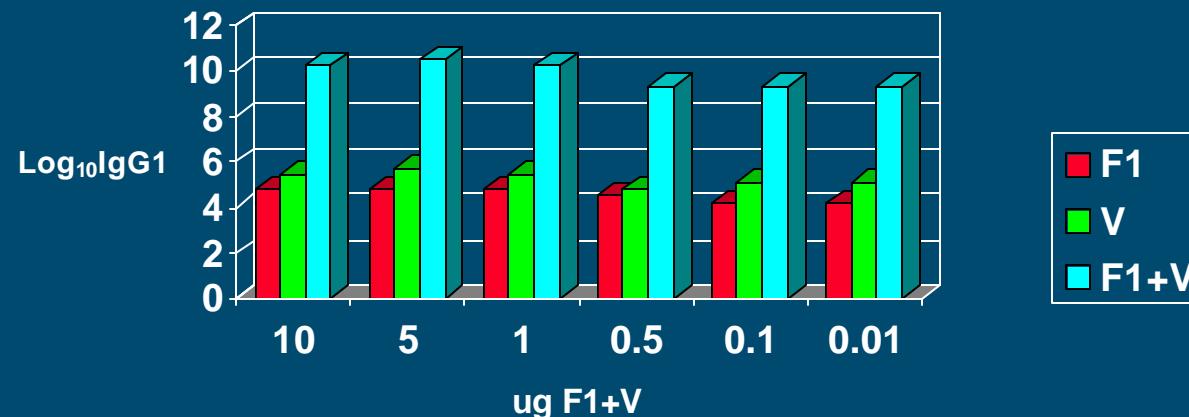
Dose-response in Balb/c mouse

Protection against s/cut challenge at d.240.

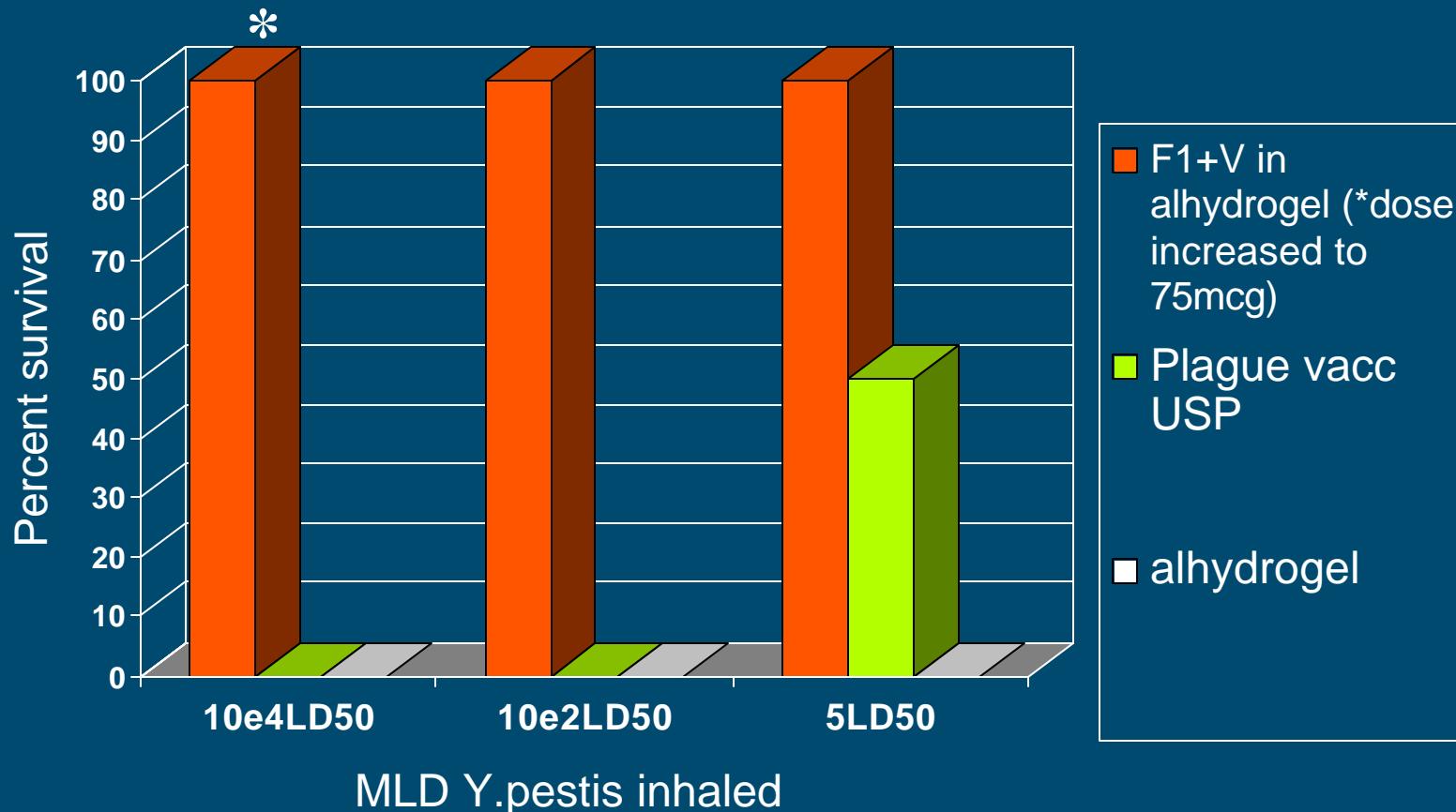
Williamson et al Clin Exp Immunol 1999, 116:107-114



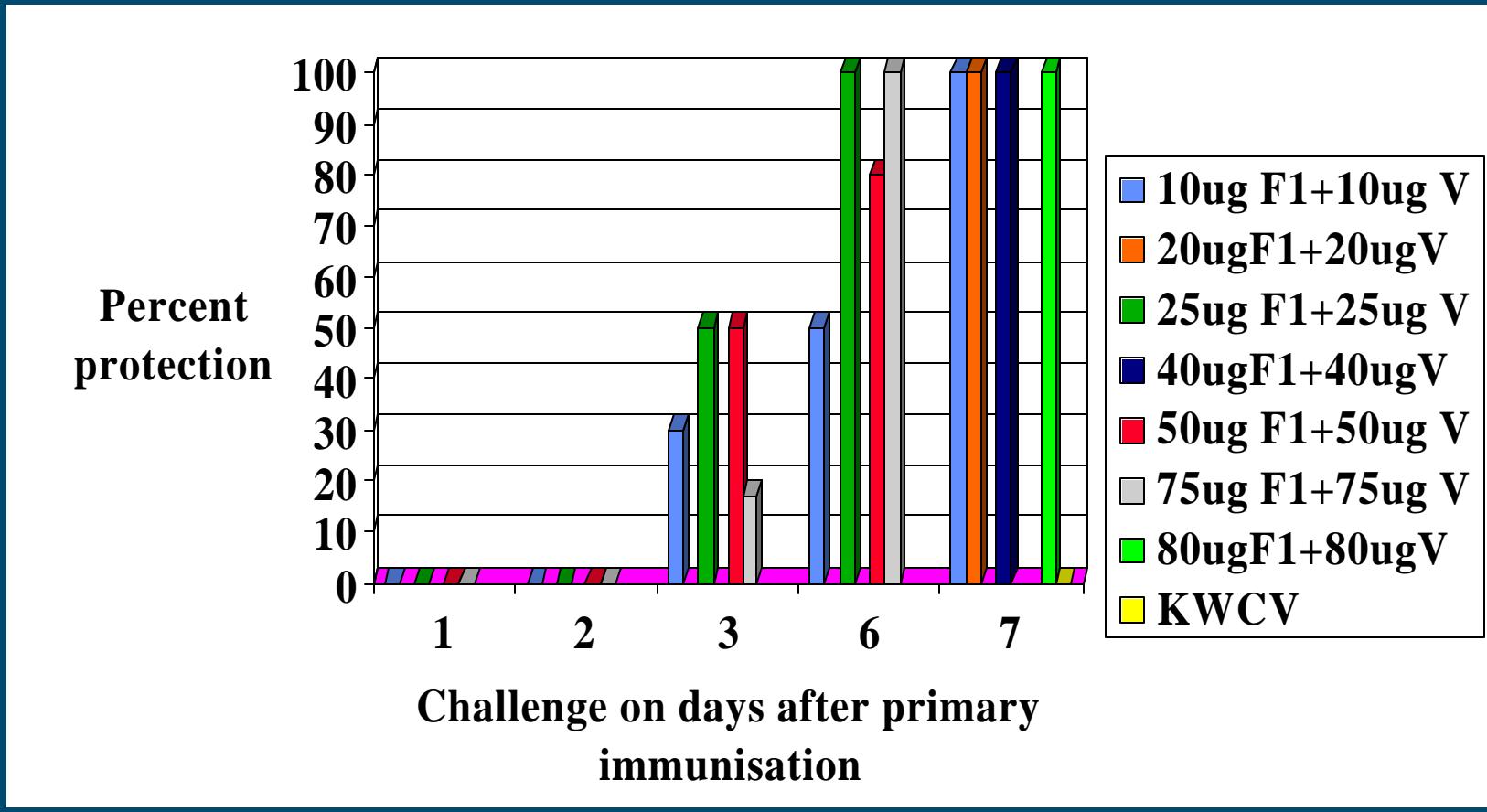
Correlation of mouse IgG1 response with protection



Protective efficacy against aerosol challenge in Porton outbred mice (Williamson et al 1997, 2001)

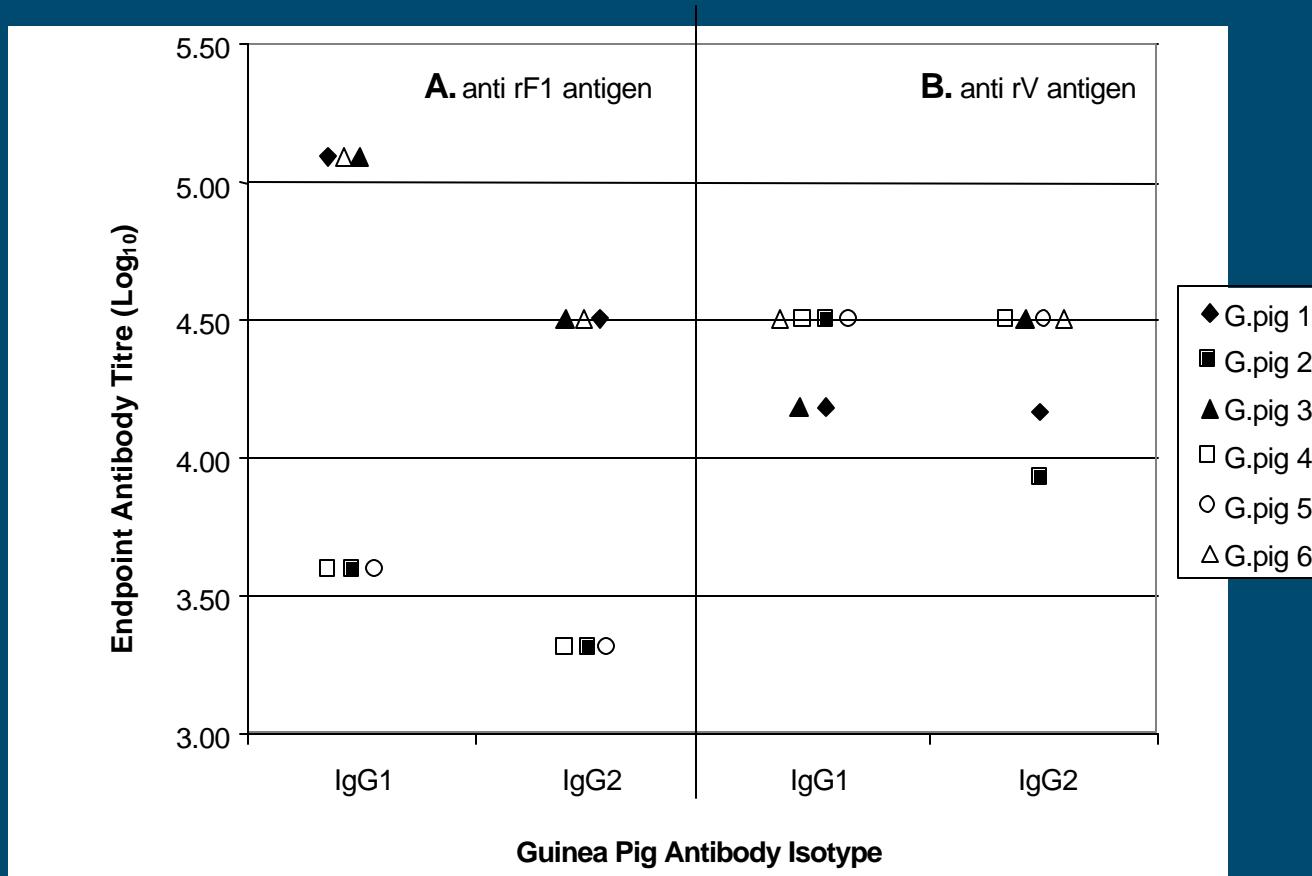


Minimum time to protection against aerosol challenge (Balb/c female mouse, 300 LD)



Antibody response in guinea pigs at challenge

(d.90) Jones et al Vaccine 2003, 21, 3912



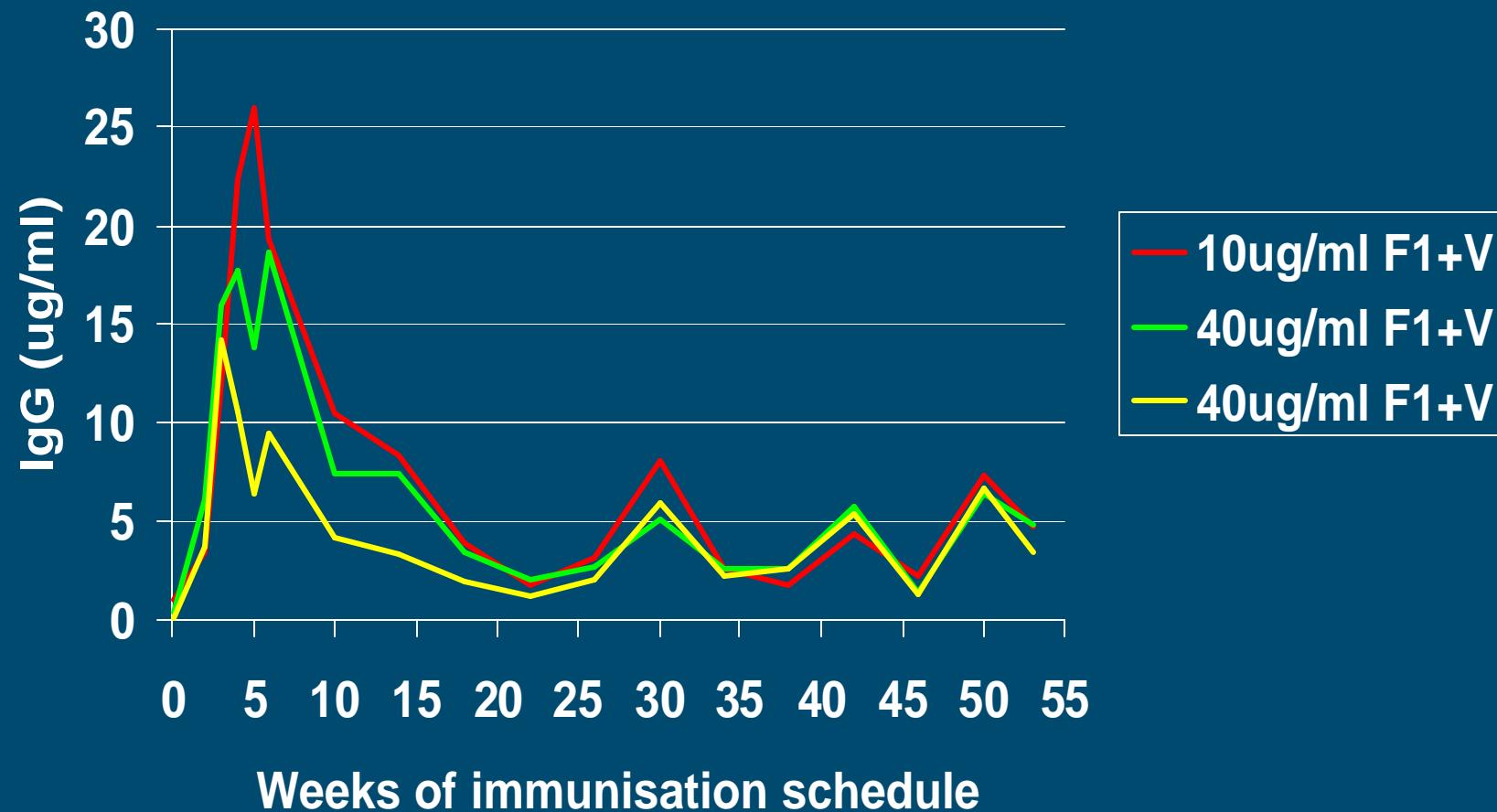
Guinea pigs

Group	Challenge dose (s.c)	Survivors	Mean ttd (days ± sem)
control	10^4	0/6	6.8 ± 0.15
rF1 + rV	10^5	6/6	
rF1 + rV	10^6	5/6	19
rF1 + rV	10^7	3/6	13 ± 1.25

Cynomolgus macaque immunisation

- Groups of 4 macaques (2 male + 2 female)
- Immunised with rF1+rV in 20% v/v alhydrogel (day 1, 21; i.m.)
- Dose levels were:
- 5mg rF1+ 5mg rV
- 10 mg rF1+ 10mg rV
- 20mg rF1+ 20mg rV
- 40mg rF1+ 40mg rV
- 40mg rF1+ 40mg rV (Single dose)

MOD051 anti-V IgG Response in Cynomolgus Macaques Immunised with sub-unit Plague Vaccine

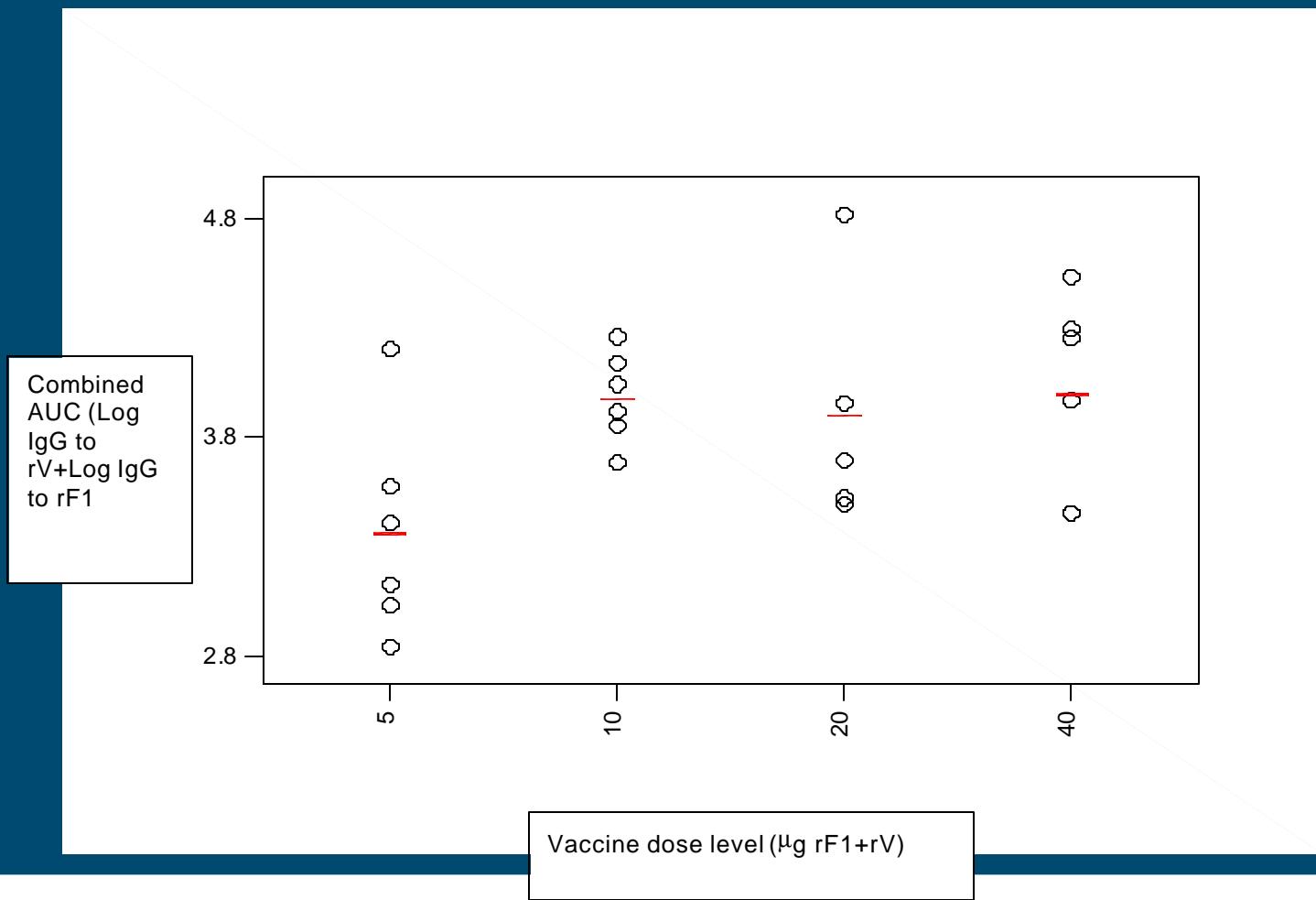


Clinical trials: Phase 1



- Ascending dose safety trial (n=32)
- 2 dose schedule- days 1 and 21(0.5ml i.m.)
- dose range 5mg rF1+ 5mg rV to 40mg rF1+ 40mg rV
- alhydrogel adjuvant
- immunogenicity in man

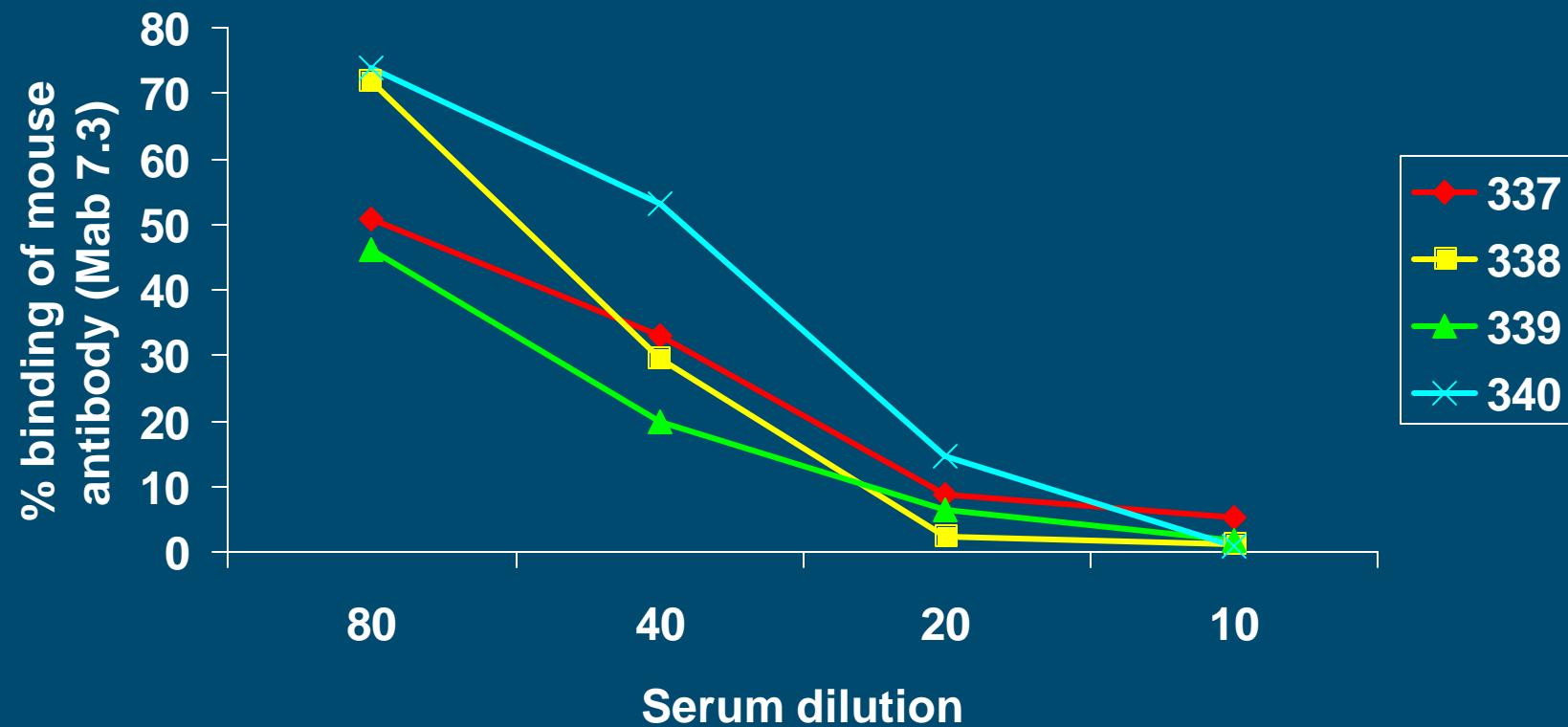
IgG response to rF1+rV in individuals across dose-levels



Antibody functionality

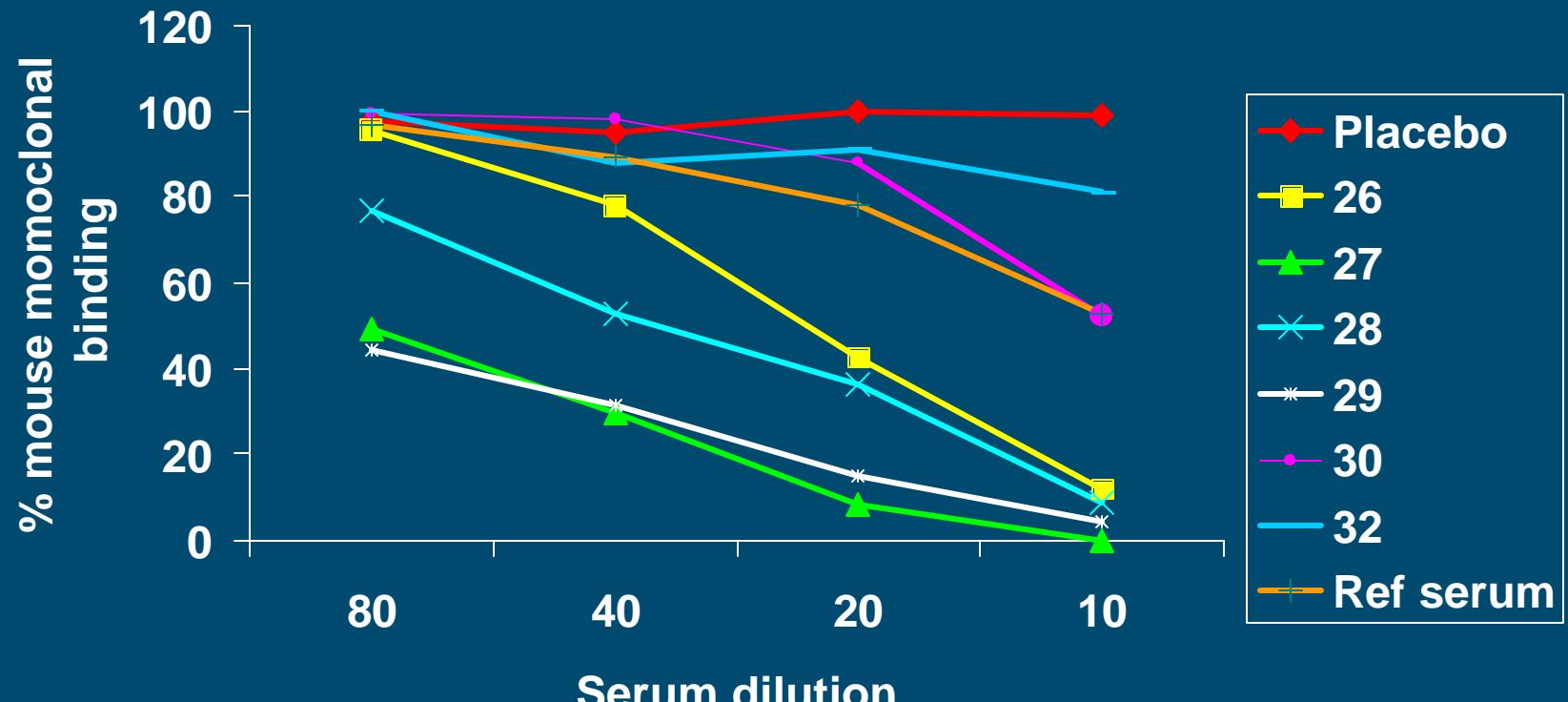
- competitive ELISA for V antigen
- inhibition of cytotoxic effect of V antigen *in vitro*
- passive transfer

Competitive Elisa for rV: Macaque serum (40mg rF1+ 40m rV) (d 35)



Competitive ELISA for rV

Human serum (40mg rF1+ 40m rV) (d 28)

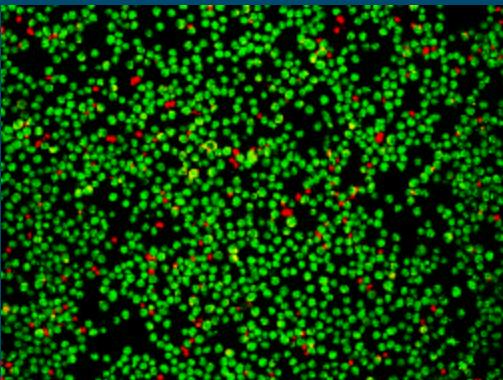


Correlation of neutralising antibody function with IgG
($r=0.79$ $p<0.001$)

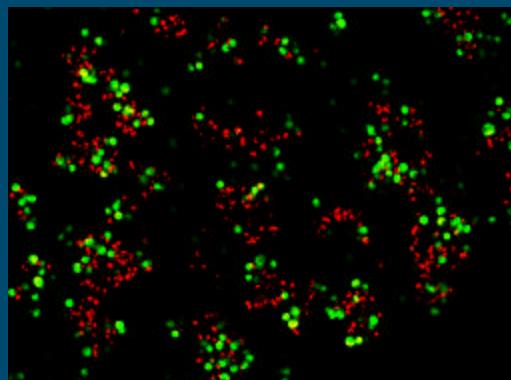
Inhibition of *In vitro* cytotoxicity

- rV expressed from *Y.pseudotuberculosis*
- Cytotoxic for macrophage cell line J774
- Look for inhibition of cytotoxicity by neutralising antibody in immune serum e.g. macaque serum
- Qualitative assay

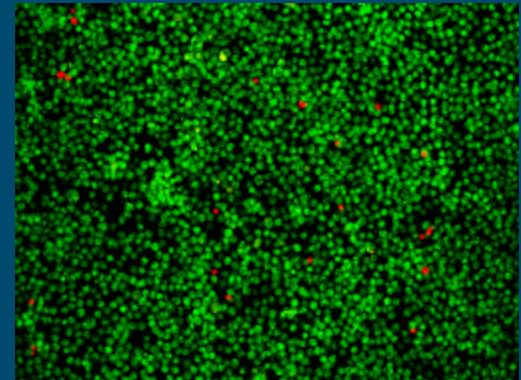
uninfected



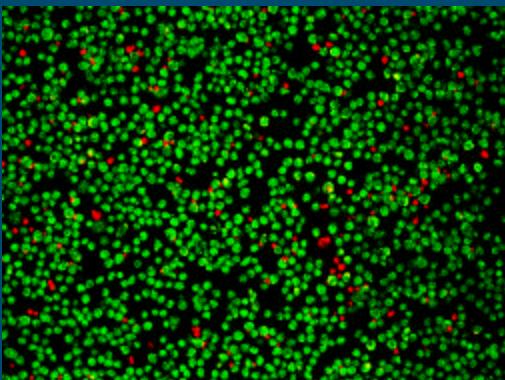
No. 3271M Day 1



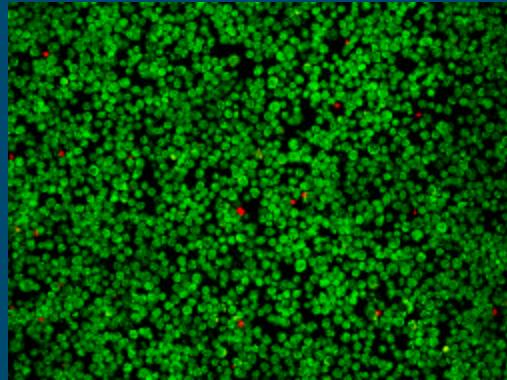
No. 344 5F Wk 6



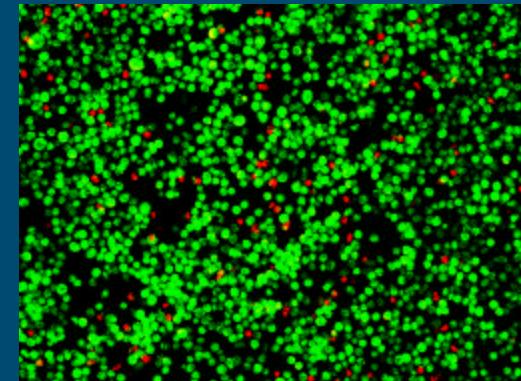
pIB19:ptrcV +mAb7.3



No. 327 1M Wk 10



No. 340 4F Wk 10



Passive transfer

Mouse

Guinea pig

Macaque

Human



mouse

Passive transfer

- Mouse to mouse
- guinea pig to mouse
- macaque

Balb/c serum protects SCID/Bge mice (Green et al 1999)

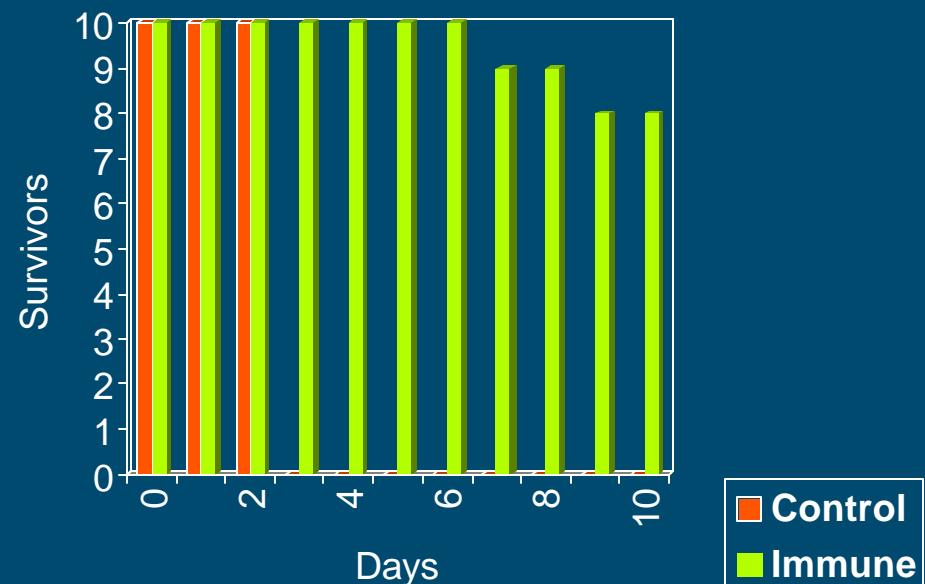
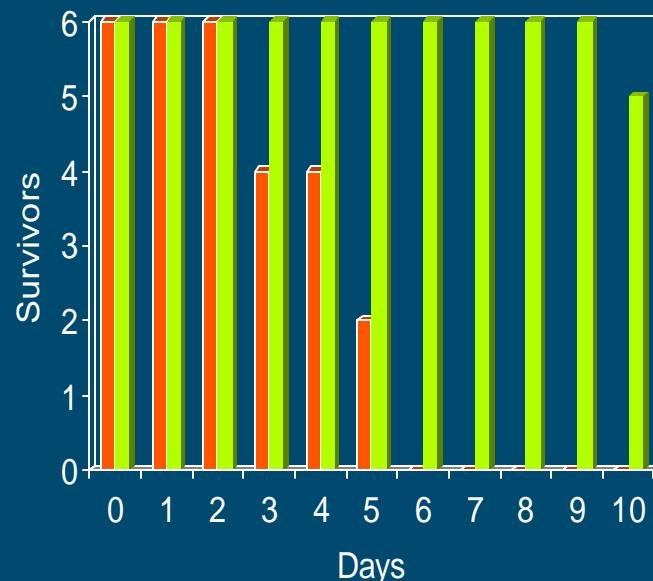


Balb/c mice (days 0, 14, 28)
with rF1+ rV vaccine

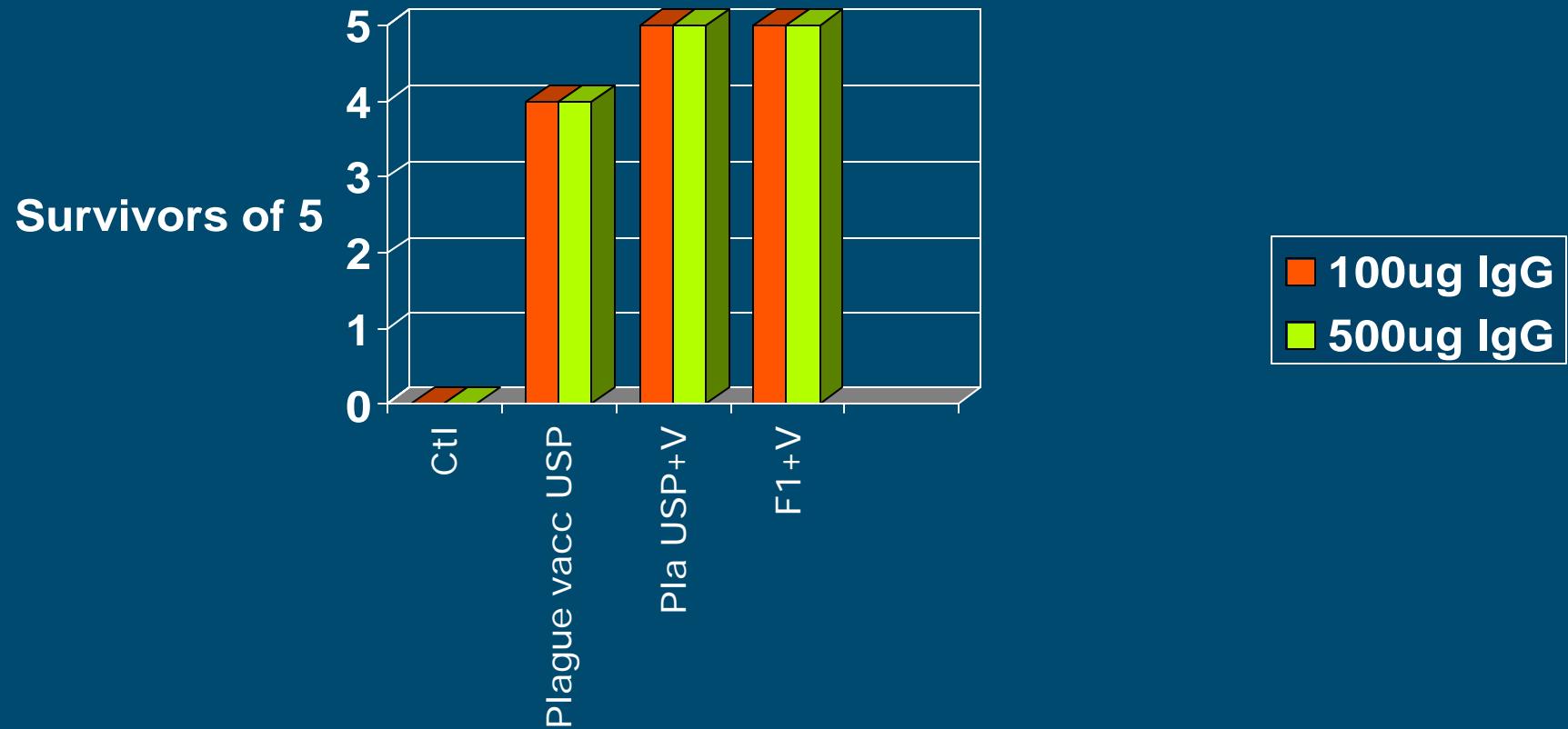
500 µl transferred I.p. to
SCID/Bge mice 20 hr
before challenge

s.c. challenge 10³ cfu

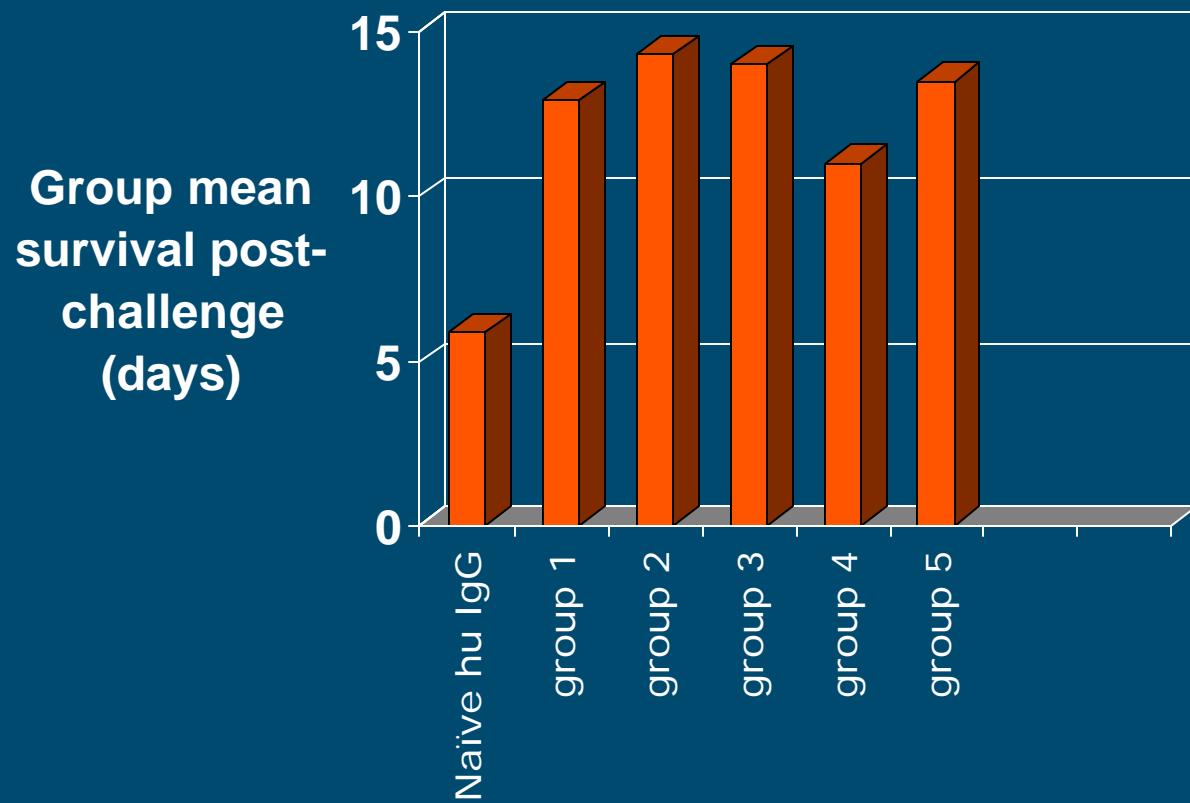
Inhaled challenge 10⁵ cfu



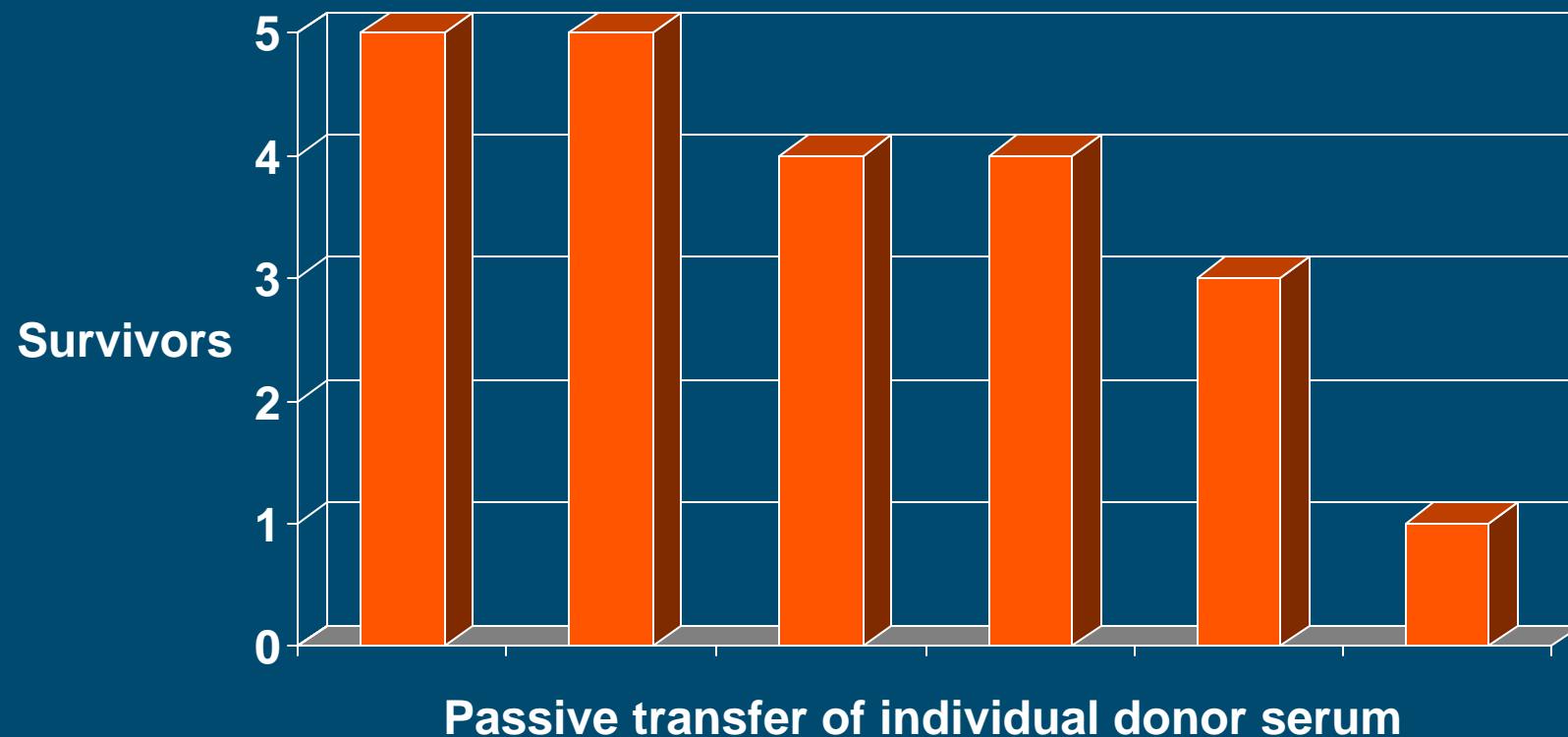
Survival of groups of mice passively immunised with 100ug or 500ug of immune guinea pig IgG against 10cfu *Y.pestis* GB (S.cut)



Survival of groups of mice passively immunised with 100ug of immune macaque IgG against 10cfu *Y.pestis* GB (S.cut)

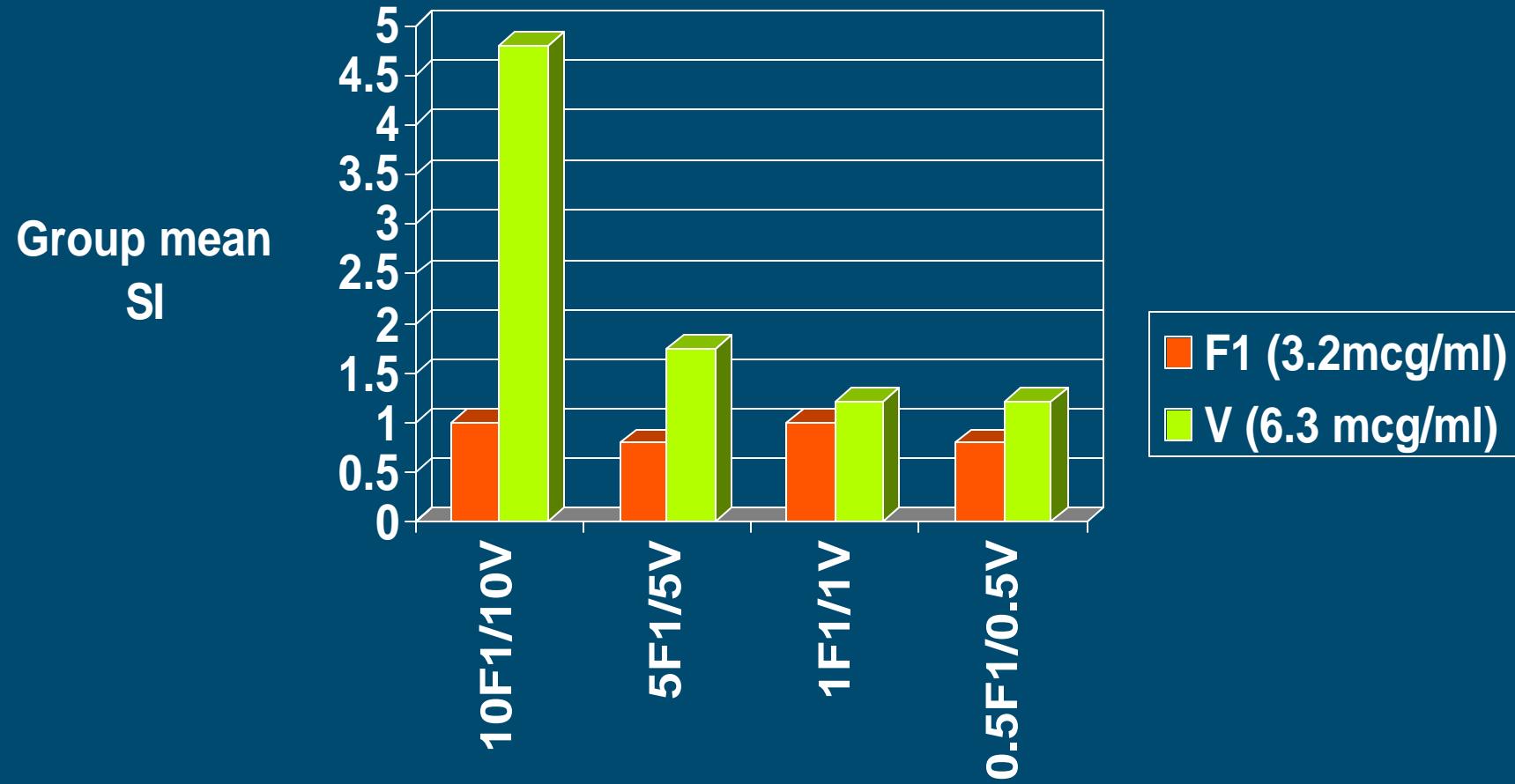


Survival of groups of mice passively immunised with immune human serum (40 mg rF1+rV) against 10cfu *Y.pestis* GB (S.cut)



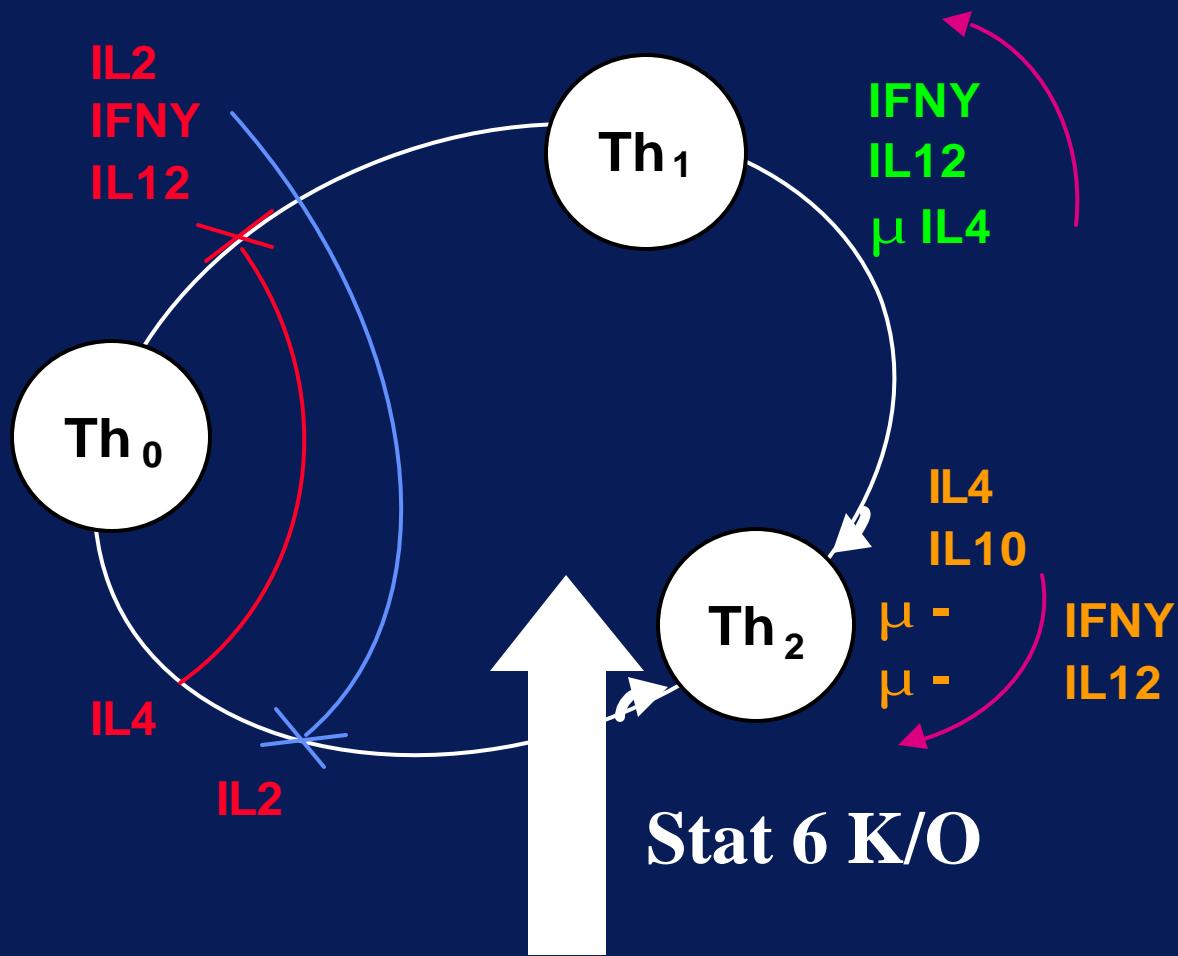
Correlation of transferable protective immunity with IgG ($r=0.73$ $p<0.01$)

CMI: T cell recall response in Balb/c mice at d.240

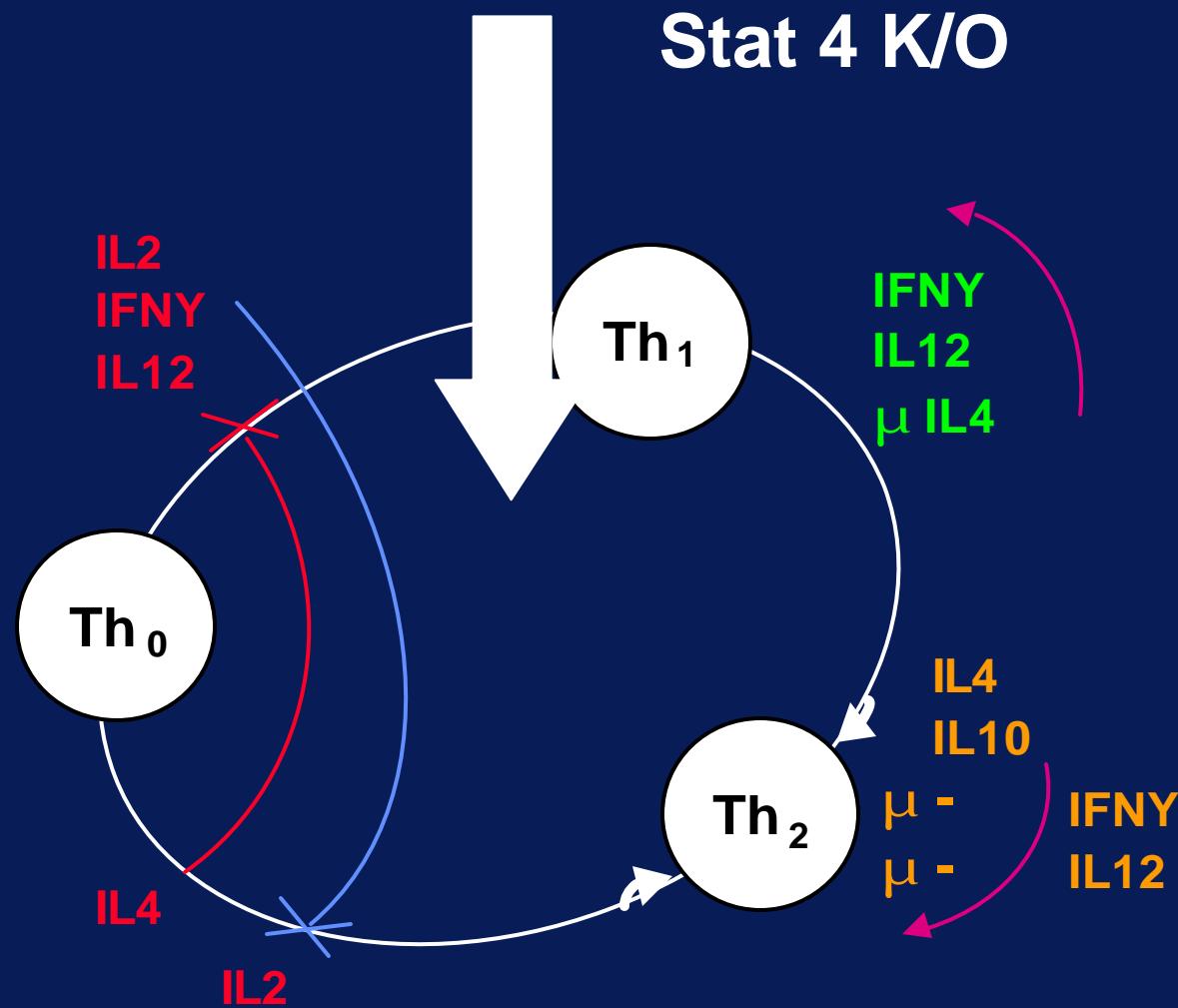


Influence of CMI

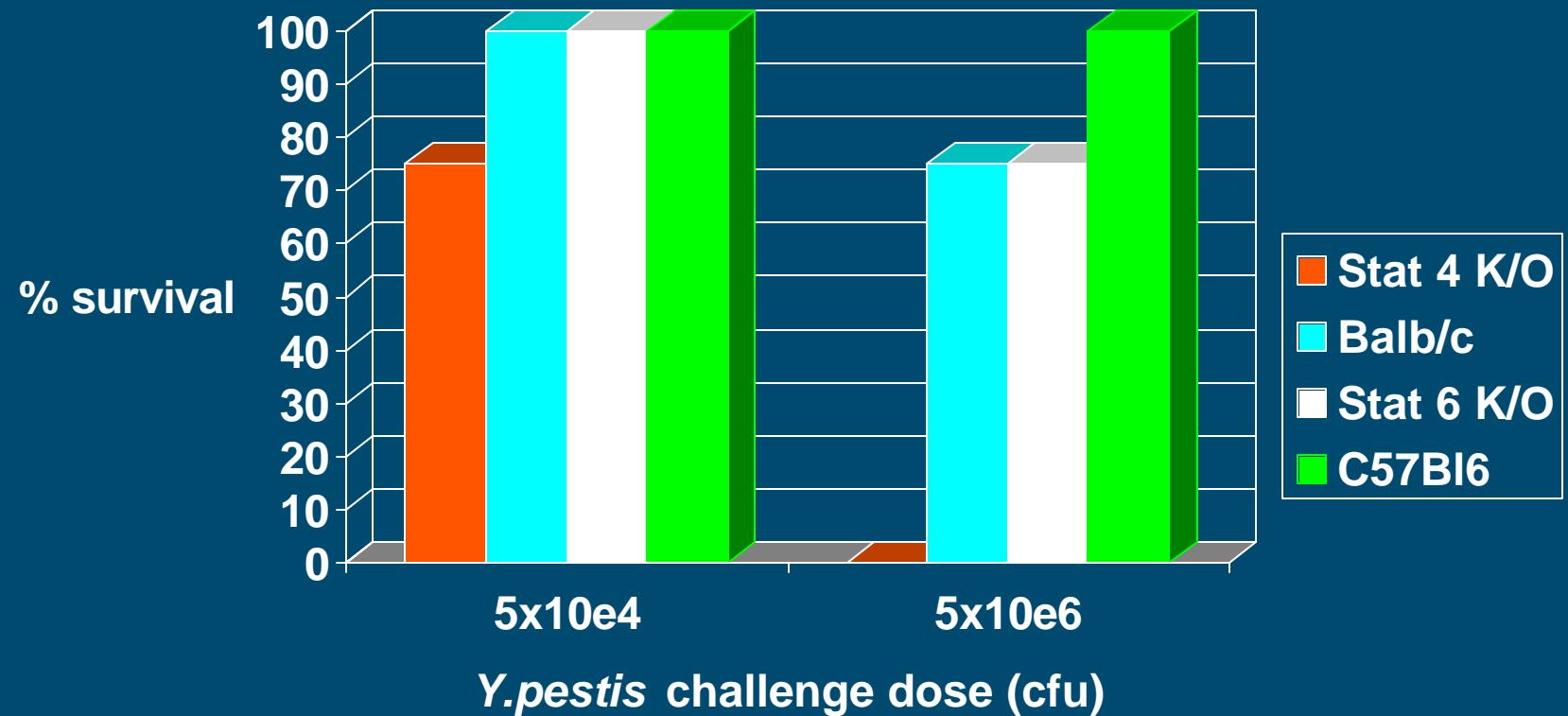
- IgG sub-classes profile in mouse, macaque and man indicates that Th2 response induced by rF1+rV vaccine
- IgG1 in the mouse correlated with protection
- Flow cytometry in mouse, macaque and man indicated a CD4+ memory response (Th2/Th1)
- But evidence from mouse models that a Th1 response to challenge is also essential to clear the infection



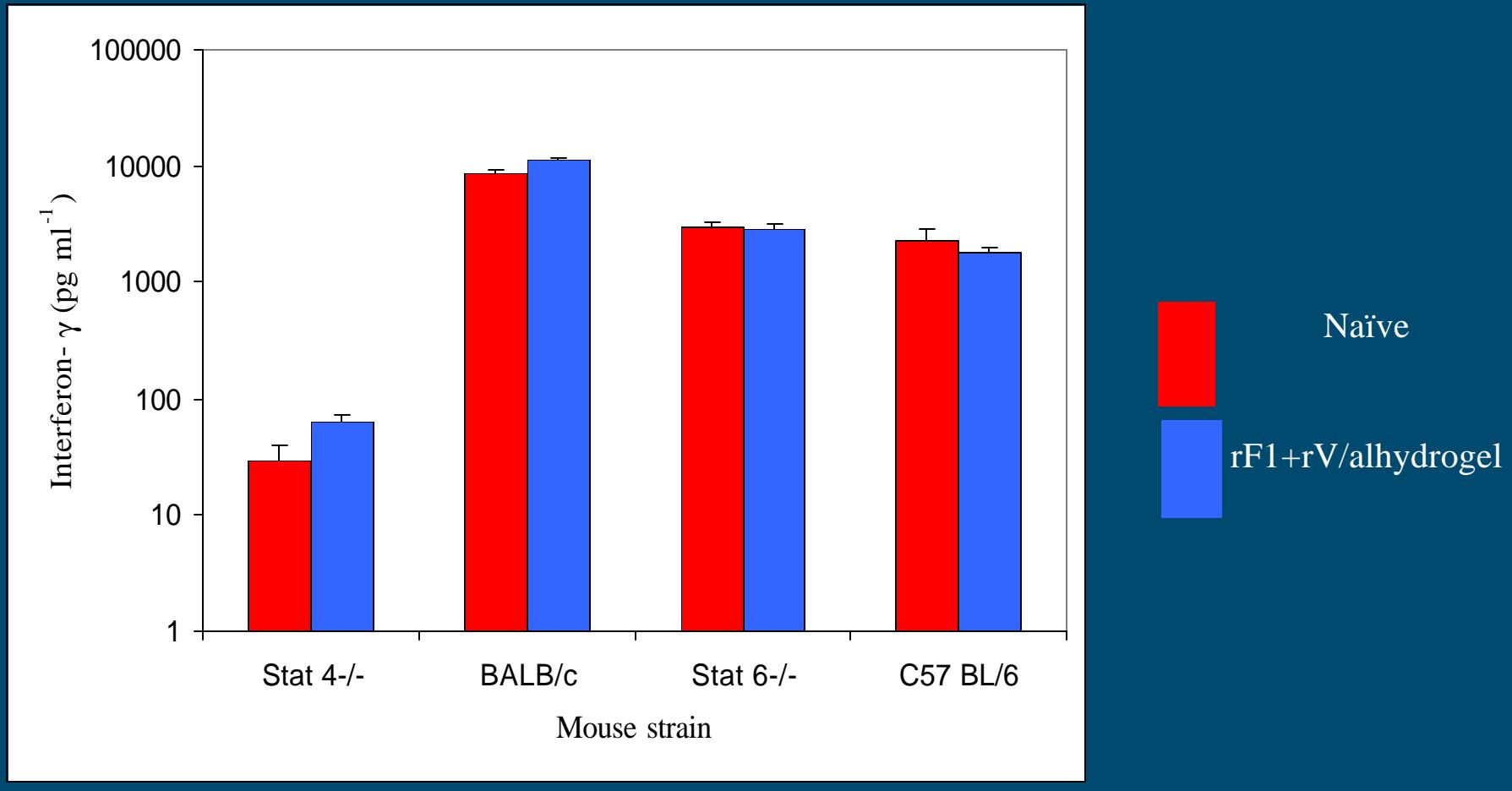
Stat 4 K/O



Reduced vaccine efficacy in Stat 4 K/O mice correlates with absence of CD4⁺ Th1 response

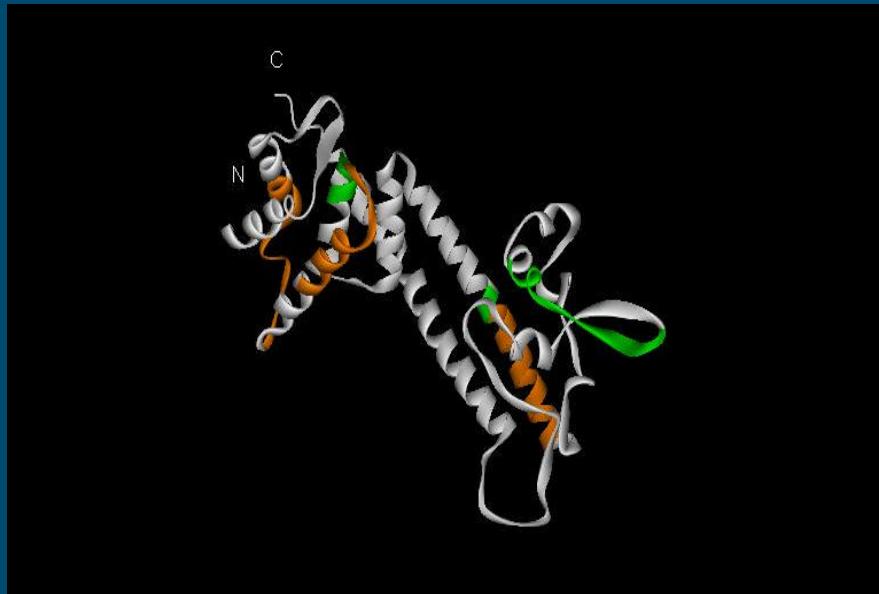


Production of IFNg by splenocytes



T cell epitopes

- Are they conserved between spp?
- Function can be attributed to murine epitopes
- Peptides comprising protective T cell epitopes provide *in vitro* targets for assay of CMI



Summary

- Antibody response conserved across species
- Antibody functionality quantifiable (CE and PT)
- Mixed Th2 / Th1 response is required to clear infection
- CMI quantifiable by *in vitro* proliferation
- Defined T cell epitopes will provide improved targets

Acknowledgments

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